Standard Operating Procedure for the Determination of Total Phosphorus, Total Dissolved Phosphorus by Persulfate Digestion and Direct Orthophosphorus Measured Without Digestion

1.0 Scope and Applicability

These methods cover the determination of the above phosphorus forms in drinking, surface and saline water, domestic and industrial wastes. The applicable range is 0.01 to 1.0 mg P/L. The curve is linear. The Method Detection Level for ortho phosphorus was found by the iterative procedure (section 17.6) and is 0.00146 mg/L the precision is 0.00057 mg/L. The Method Detection level for the persulfate digestion method is 0.014 mg/L and the precision is 0.005 mg/L. This is for a single operator on a single day. These detection levels can be compared with actual Performance levels (PDL) section 14.0. (See EPA chart, section 18.0, for more information on phosphate forms).

2.0 Summary of Method

The persulfate digest is the weakest of the acid digests which convert the various forms of phosphorus to orthophosphate. More rigorous digestions which employ perchloric acid or nitric acid-sulfuric acid are required for difficult samples but are not presently being used in our lab. Results when compared between the persulfate digest and the total phosphorus obtained from the TKN digest (17.1) may not agree completely due to the more rigorous TKN digestion. The ortho phosphate produced as a result of the digestion step is reacted with ammonium molybdate and antimony potassium tartrate in an acid medium to form an antimony-phosphomolybdate complex. The complex is reduced to a blue-colored complex by ascorbic acid. Absorption is measured at 880 nm. Ortho Phosphate is measured directly without a digestion step and within 48 hours. The addition of H_2SO_4 to preserve the sample for a longer holding time would also digest some of the organic forms, thus complicating an analysis for only ortho PO4.

3.0 Definitions

3.1 Total Phosphorus: all of the phosphorus present in the sample, regardless of form, as measured by the persulfate digestion procedure.

3.2 Dissolved Phosphorus: All of the phosphorus present in the filtrate of a sample passed through a 0.45 micron filter measured by the persulfate digestion.

3.3 Orthophosphate: Inorganic Phosphorus (PO_4^{-3}) measured by direct colorimetric analysis without digestion.
A complete break down of the Phosphorus forms will be found in section 18.0.

4.0 Interferences

4.1 Arsenate is determined similarly to phosphorus and should be considered when present in quantities approaching that of the phosphorus. It can be reduced to arsenious acid with sodium bisulfite.

4.2 High concentrations of iron will use some of the ascorbic acid leading to low recovery of phosphorus. This should not be a big problem with the high concentration used in the ascorbic acid solution.

4.3 Glassware contamination is a problem at low levels. Glassware should be rinsed in 1:1 HCl when problems are suspected. The glassware should be devoted to the PO4 digestions. Eliminate the use of soaps containing phosphates.

4.4 Silica forms a pale blue complex which also absorbs at 880 nm. This shouldn't be a problem as 30 mg SiO_2/L is required to produce a positive error of 0.007 mg P/L.

4.5 A separate helium degassing line should be devoted to the PO_4 chemistry as many reagents for other analytes contain phosphate buffers.

5.0 Safety:

There are no known safety hazards associated with this method other than normal laboratory practices. This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material safety data sheets (MSDS's) should be available to all personnel involved in these analyses.

6.0 Equipment and Supplies:

Note: Brand names, suppliers, and part numbers are cited for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using equipment and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.

6.1 Lachat QuikChem 8000 Flow Injection Analyzer

6.1.1 XYZ autosampler

6.1.2 Proportioning pump

6.1.3 Injection module

6.1.4 Colorimeter with: 10 mm flow cell,
880 nm interference filter, and 90 cm loop

6.1.5 Phosphorus reaction module

6.1.6 Gateway E-4200 computer

6.1.7 EV700 Monitor

6.1.8 Omnion Software Ver 2.0 (Jan 1999)

6.1.9 HP 8150 printer

6.1.10 Heater set to 37 deg C, with 175 cm of tubing

6.1.11 Corning Hot Plate

6.1.12 125 ml Erlenmeyer flasks

7.0 Reagents and Standards:
All reagents are ACS Reagent grade or higher.

Reagents:

7.1 Stock ammonium molybdate solution
In a 1L volumetric flask dissolve 40.0 g of ammonium molybdate tetrahydrate $[(NH_4)_6Mo_7O_{24}\bullet 4H_2O]$ in approximately 800 ml of water. Dilute to the mark and invert to mix. Store in plastic and refrigerate.

7.2 Stock antimony potassium tartrate solution
In a 1L volumetric flask, dissolve 3.0 g of antimony potassium tartrate $[K(SbO)C_4H_4O_6\bullet 1/2H_2O]$ in approximately 800 ml of water. Dilute to the mark and invert to mix. Store in a dark bottle and refrigerate.

7.3 Molybdate color reagent
Add about 500 ml of water and 20 ml of concentrated sulfuric acid (H_2SO_4) to a liter beaker. Stir to mix. When cool, add 213 ml of stock ammonium molybdate solution and 72 ml of stock antimony potassium tartrate solution. Dilute to 1L. Degas with helium using a separate PO_4 line. This reagent is stable for 3 weeks.

7.4 Ascorbic acid reducing solution

In a 1L beaker dissolve 60.0 g ascorbic acid and 1.0 g of dodecylsulfate, sodium ($CH_3(CH_2)_{11}OSO_3Na$) in about 700 ml of water. Dilute to the mark and invert to mix. This solution is stable for two weeks.

7.5 Sulfuric acid solution (11 N)
Cautiously add 305 ml concentrated H_2SO_4 slowly and with stirring to 600 ml of deionized water. Cool by use of a plastic bucket and ice. Bring to a final volume of 1 liter.

7.6 Carrier
Direct orthophosphate: Use degassed distilled water. Persulfate digestion: Add 6.1 ml concentrated H_2SO_4 to 800 ml deionized water. Dissolve and dilute to 1L. Degas with helium.

7.7 Sodium hydroxide - EDTA rinse
Dissolve 65 g of sodium hydroxide (NaOH) and 6 g of tetrasodium ethylendiamine tetraacetic acid in 1L of water.

7.8 Ammonium persulfate ($(NH_4)_2S_2O_8$)

Standards

7.9 Stock standard (100.0 mg P/L)
In a 1L volumetric flask place 800 ml water and 0.4398 g of anhydrous potassium dihydrogen phosphate (KH_2PO_4) (NBS 200) which has been previously dried at 105 deg C for 1 hr. Dilute to the mark and invert to dissolve.

7.10 Working orthophosphate standards
Pipette the indicated amount of stock standard (8.9) into a 100 ml volumetric flask and dilute to volume with deionized water.

ml stock	mg P/L
1.000	1.000
0.700	0.700
0.400	0.400
0.250	0.250
0.100	0.100
0.030	0.030
0.010	0.010
0	0

8.0 Sample Collection, Preservation and Storage

Samples not analyzed immediately (within 48 hrs) after collection should be preserved with H_2SO_4 to a pH <2 and refrigerated at 4 deg C. Samples for direct ortho analysis should not be preserved but should be refrigerated. EPA procedures approve the use of plastic containers for samples to be analyzed for phosphorus. Standard Methods suggest that at low levels (not defined but probably <1 ppm like river samples), phosphorus can be absorbed by the walls of plastic containers and therefore should not be used. It is conceivable that our present procedure may give a negative bias to the results.

9.0 Quality Control

9.1 Each sample run will include 1 check sample, 1 duplicate and 1 spike sample per 10 samples run on the FIA.

9.2 The check sample, as well as the standards, will be used throughout the day that phosphorus is run and will be digested only once.

9.3 Ortho PO4 spikes are typically 0.3 ppm (0.03 ml of 100 ppm spike in 10 ml soln.) and persulfate spikes are typically 0.4 ppm (.2 ml of 100 ppm in 50 ml soln.)

9.4 The check, spike and duplicate samples will be reviewed. Corrective action will be taken if variations, from statistical expectations, can't be explained. The expectation is a 10% variation or less on LFB, and check samples.

10.0 Calibration and Standardization:

Standardization is achieved using Lachat's Omnion software ver 2.0. All standard curves are linear and will run with a correlation coefficient of 0.995 or better. Failure at this point will require a re-standardization or preparation of new standards. Typical standardization curves are listed in section 18. All data stored with the Omnion software used on the Quik-Chem 8000 provides for imbedding of the calibration curve in the data file. The electronic data file including the chromatograms can be retrieved and reviewed. The file can be reprocessed with new parameters but the original data will remain unchanged.

The instrument will use 5 outside calibration standards and a zero for each run made as outlined in section 12.1.5. In addition the high standard will be checked at intervals of 25 samples for calibration drift. A change in 8% will initiate a re-calibration and continuation. A change of 10% will initiate a re-calibration and a rerunning of all samples since the last calibration. This re-calibration is under software control.

11.0 Procedures

11.1 **Digestion Procedure for Persulfate digests**

11.1.1 Add one glass boiling bead and 0.4 g ammonium persulfate (two level measuring spoon) to a 125 ml Erlenmeyer flask.

11.1.2 Transfer 50 ml of sample or an aliquot diluted to 50 ml, using a graduated cylinder, to the flask. Add 0.200 ml of 100 ppm orthophosphate spike solution to spikes. This will provide a 0.4 ppm spike. Add 1 ml of 11 N sulfuric acid H2SO4. Mix.

11.1.3 Place flasks on hot plate set on high and boil until 10-20 ml are left.

11.1.4 Remove flasks from hot plate and cool. Bring sample back to 50 ml using a 50 ml graduated cylinder and deionized water.

11.1.5 **Persulfate standards** should be run through the digestion like samples. Use the following amount of stock orthophosphate standard in their preparation. Add 50 ml of deionized water to each flask.

ml Stock	ppm P/L
0.500	1.000
0.250	0.500
0.050	0.100
0.025	0.050
0	0

11.1.6 Analyze on FIA

11.2 Procedure for ortho phosphate analysis:

Samples are poured into tubes with no additional treatment. Standards are made according to section 8.10. The Standards are: 1, 0.7, 0.4, 0.25, 0.10, 0.03, 0.01, 0 ppm phosphate.

11.3 FIA Procedure

11.3.1 Place both the orange-white and Carrier (red) lines in carrier solution.
Ortho carrier is DI water
Persulfate carrier is sulfuric acid/potassium sulfate solution.

11.3.2 Install the phosphate manifold, run the feed lines through the pump cassette, and snap it into place. Turn on the pump. Place all feed lines

into distilled water. Check for leaks.

11.3.3 Turn on the other modules.

11.3.4 Place standards and samples into the auto sampler using the work list format.

11.3.5 Place all feed lines into proper reagent containers.

11.3.6 Call up the proper method on the FIA computer.

11.3.7 Start analysis when the baseline is stable.

11.3.8 At the end of the run, rinse all feed lines for 5 min in EDTA rinse solution. Then 5 min in deionized water. Pump the lines dry.

11.3.9 Turn off the pump and all modules. Release the pump tube cassettes.

12.0 Data Analysis

Calculations will be made by the FIA computer using a least squares linear regression on the standards. Results will be reported in mg P/L to three decimal places.

13.0 Method Performance:

The following method detection levels (MDL), and precision were obtained by spiking deionized water with low levels of a phosphorus standard. The performance detection levels (PDL) for analyte {9412} were obtained from our data base on 33 duplicates over the range of 0.01 to 0.4 mg P/L. The PDL for analyte {9415} were obtained from our data base on 19 duplicates over the range of 0.018 to 0.10 mg P/l. Detection level documentation is provided in section 18.

DETECTION LEVELS

Analyte #	PDL	precision(σ)	MDL	precision(σ)
9408	limited data		0.00057	0.000213
9410	0.0163	0.00635	0.00057	0.000213
9412	0.0257	0.0102	0.01435	0.00509
9415	0.0504	0.0197	0.01435	0.00509

14.0 Pollution Prevention:

We have no knowledge of any pollution associated with this method.

15.0 Waste Management:

This method tends to have a problem with the molybdate reagent expiring before it is used up. Early signs of deterioration are a blue precipitate in the manifold tubing and a white deposit at the bottom of the reagent bottle. This creates an extra waste disposal problem for reagent 9.3. The minimum amount needed of this reagent should be prepared.

For further information on waste management consult the Waste Management Manual for Laboratory Personnel and Less is Better: Laboratory Chemical Management for Waste reduction both available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

16.0 References

16.1 Lachat QuikChem Method (Sept 94) 10-115-01-1-A ortho P

16.2 Lachat QuikChem Method (May 92) 10-115-01-1-E (persulfate hot place digest)

16.3 EPA (Aug 93) Method 365.1 (Determination of Phosphorus by semi-automated colorimetry)

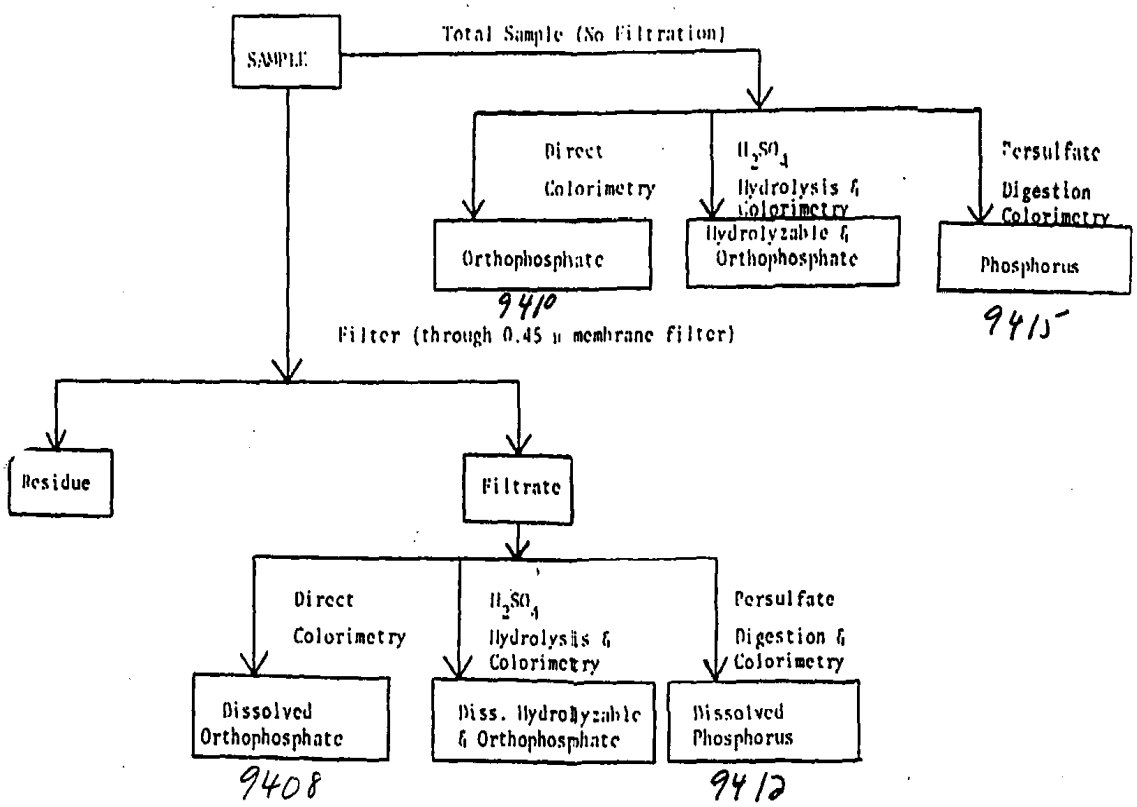
16.4 EPA Appendix B to Part 136 - Definition and Procedure for the Determination of the Method Detection Limit - Revision 1.11, 40 CFR Ch. 1 (7-1-94 Edition).

17.0 Tables, Diagrams, Flowcharts, Validation Data and Additional Information

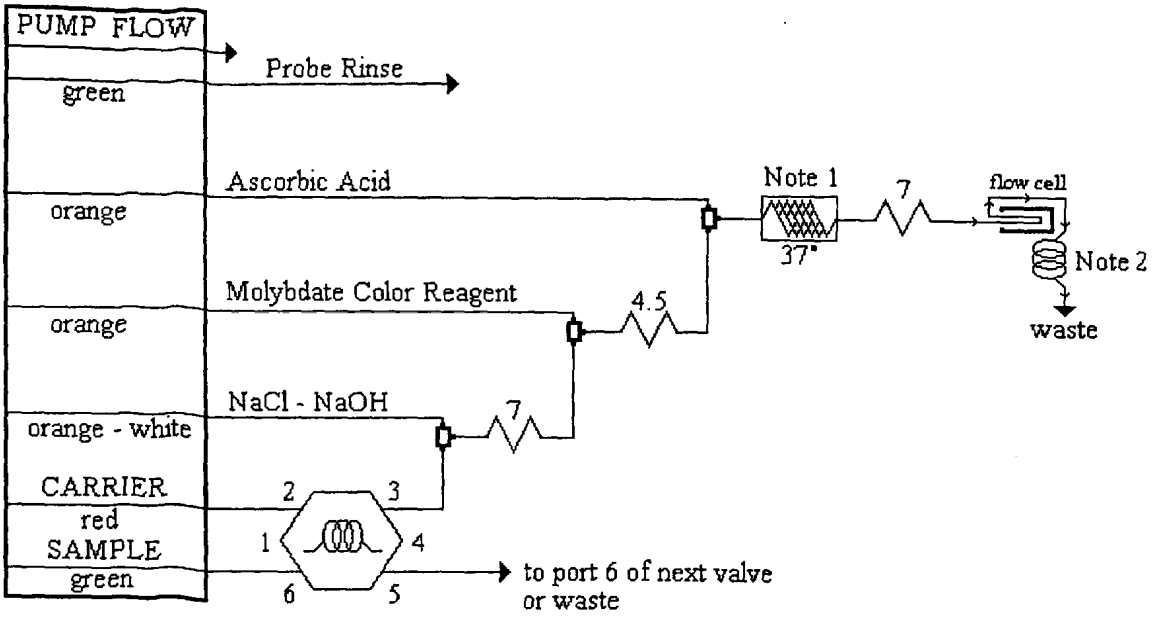
TYPES OF PHOSPHORUS

Analyte #	analyte	Description: Persulfate .45 µ filter	Description: Persulfate digest
9408	dissolved ortho PO4	Yes	No
9410	total ortho PO4	No	No
9412	total dissolved PO4	Yes	Yes
9415	total PO4	No	Yes

Figure 1. Analytical scheme for differentiation of phosphorus forms (17.5).



Total Phosphorus and Ortho Phosphorus Manifold Diagram

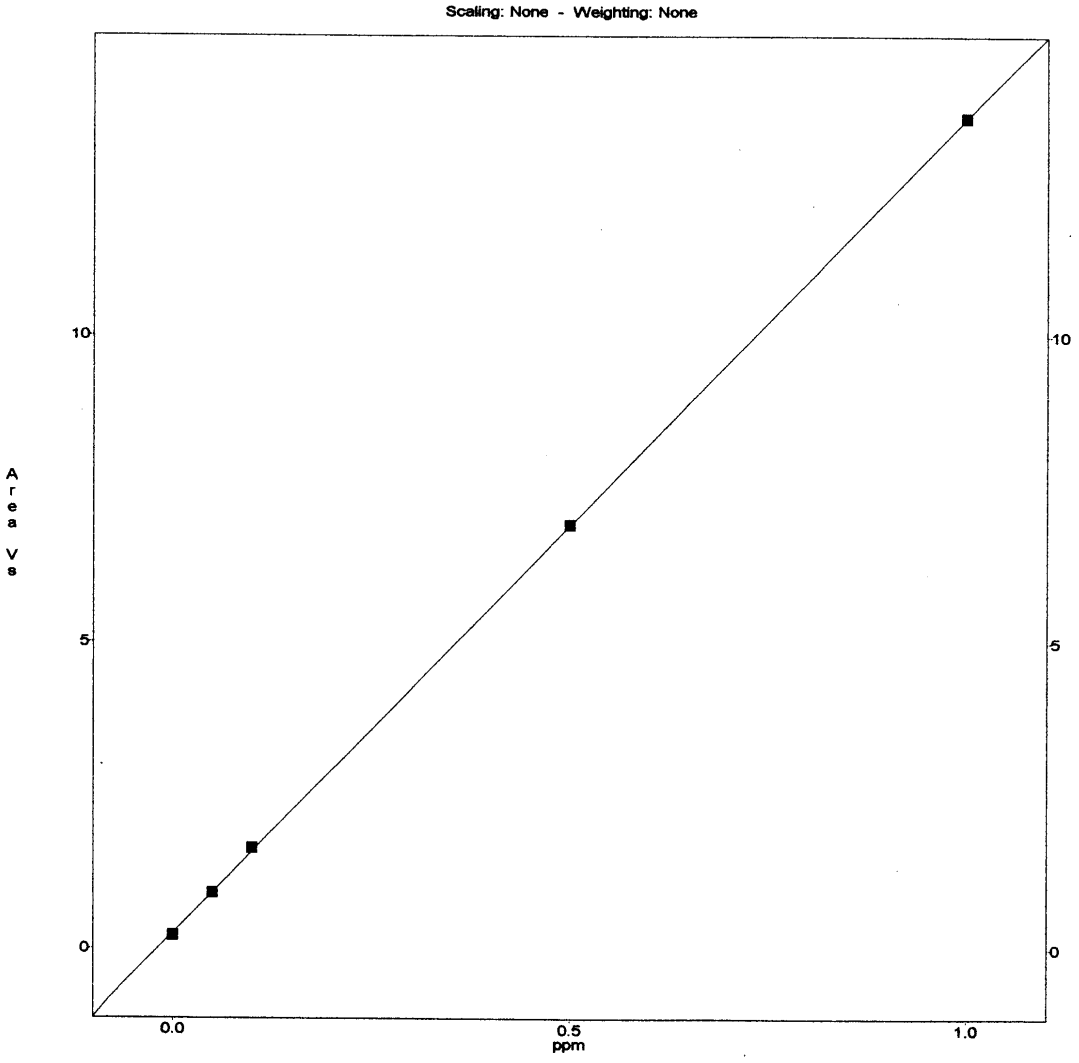


4.5 is 70 cm of tubing on a 4.5 cm coil support
7 is 135 cm of tubing on a 7 cm coil support
Apparatus: Standard valve, flow cell, and detector head modules are used.
All manifold tubing is 0.8 mm (0.032 in) i.d. This is 5.2 μL/cm.
Note 1: 175 cm of heated tubing
Note 2: 2 meter restrict or coil, 0.52 mm (0.022 in) i.d.

PPM PO4

Lvl	Area	ppm	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Replic STD	Replic % RSD	Residual 1st Poly
1	13569218	1.00	13569218					0.0	0.0	0.1
2	6924322	0.50	6924322					0.0	0.0	-0.1
3	1648258	0.10	1648258					0.0	0.0	-4.2
4	918485	0.05	918485					0.0	0.0	1.3
5	217608	0.00	217608					0.0	0.0	

1st Order Poly
Conc = 7.510e-008 Area - 1.963e-002
R^2 = 1.0000



Printed: Thursday, February 15, 1996 - 03:16 PM

Typical ortho Phosphorus Calibration Curve

OPERATOR: diane
ACQ. TIME: Feb 15, 1996 15:09:39
DATA FILENAME: C:\OMNION\DATA\9602150G.FDT
METHOD FILENAME: C:\OMNION\METHODS\PER_SO4.MET
TRAY FILENAME: C:\OMNION\TRAYS\PER_SO4.tra

Feb 15, 1996

Multi-Channel Table
Type: Unknowns
Channel Range: 2 to 2 -- Cup Range: 1 to 40

Cup	Log number	# of Reps	PPM PO4 (ppm)	Man Dil Factor	Auto Dil Factor
1	.02	1	0.01413	1.0	1.00
2	.02	1	0.01295	1.0	1.00
3	.02	1	0.01986	1.0	1.00
4	.02	1	0.02619	1.0	1.00
5	.02	1	0.01574	1.0	1.00
6	.02	1	0.02267	1.0	1.00
7	.02	1	0.01718	1.0	1.00
8	.02	1	0.01948	1.0	1.00
9	.02	1	0.01339	1.0	1.00
10	.02	1	0.02683	1.0	1.00

$\bar{x} = .018842$
$\sigma = .0050873$
$n = 10$

S/N ratio $\bar{x}/\sigma$
$= .018842/.005087 = 3.70$

MDL $= (.0050873)(2.821)$
$= .01435$

S/N ratio is between 2.5 and 5.0
spk is between MDL and 10 MDL
recovery of spk is $.018842/.02 \times 100 = 94.2\%$, seems acceptable
There does not appear to be any outliers in the data set
There is no required MDL (SDWA + Lust)

Method Detection Level data for ortho Phosphorus

Method Detection Level data for total Phosphorus by persulfate digest